REMARKS

Upon entry of this amendment, claims 32, 36-39, 42, 46-49, 52-55, 84 and 89-90 are pending. Claims 32, 36, 42, 46, 52-54, 84 and 89 have been amended. Support for these amendments can be found in as-filed claim 52 and the specification at, *e.g.*, page 4, lines 26-29; page 9, lines 27-28; and page 21, lines 10-24. The specification has been amended to replace the pending formal drawings with replacement formal drawings for Figures 1-13. No new matter has been added.

Objections to the Specification

The Examiner has objected to the disclosure because Figure 13, which is indicated in the Brief Description of the Drawings, was missing from the February 12, 2001 submission and Figures 11B-13 were missing from the formal drawings submitted on July 19, 2001. In response, Applicants file herewith replacement formal drawings for Figures 1-13. Applicants also submit a copy of the stamped return postcard filed with the February 12, 2001 application which indicates that Figures 1A-13 were filed with the original application and were received by the USPTO. Therefore, this objection has been overcome and should be withdrawn.

Claim Rejections -- 35 U.S.C. § 112, second paragraph

1. Claims 32, 35-39, 42, 45-49, 52-55, 84, 89 and 90 have been rejected as indefinite. The Examiner has indicated that the metes and bounds of the amended claims containing the term "cytotoxic immune effector cells" are uncertain, since it is unclear what cell types the term includes or excludes. Claims 35 and 45 have been cancelled herein. Thus, this rejection is moot with respect to these claims. Applicants have amended claims 32, 42, and 84 to specify that the population of educated, antigen-specific immune effector cells is cytotoxic. Thus, Applicants contend that the metes and bounds of these claims (and their respective dependent claims) is clear. Therefore, this rejection should be withdrawn.

2. The Examiner has also indicated that the pending claims are vague and indefinite because of the use of the term "substantially pure population of educated, antigen-specific cytotoxic immune effector cells expanded in culture," as found in claim 32. Applicants have herein amended claim 32 to specify "[a] substantially pure cytotoxic population of educated, antigen-

specific immune effector cells expanded in culture by contacting immune effector cells with hybrid cells, wherein the immune effector cells are T lymphocytes..." Thus, the immune effector cells of amended claim 32 do not "encompass a diverse population of cells such as B cells, monocytes, macrophages, NK cells and any T lymphocytes (e.g., CD3+, CD4+, or CD8+)" as asserted by the Examiner. (Office Action, paragraph bridging pages 3 and 4). Rather, the population of immune effector cells is now limited to T lymphocytes. Further, Applicants also note that the specification teaches that "[the] antigen-specific immune effector cells are expanded at the expense of the hybrid cells, which die in the culture." (Specification at page 25, lines 2-3). Therefore, the resulting cell population would be a substantially pure cytotoxic population of educated, antigen-specific T lymphocyte immune effector cells, as recited by amended claim 32. Thus, this rejection has been overcome and should be withdrawn.

Claim Rejections -- 35 U.S.C. § 112, first paragraph

1. Claims 32, 35-39, 42, 45-49, 52-55, 84, 89 and 90 are rejected for lack of enablement. The Examiner states that, "[g]iven the broadest reasonable interpretation in light of the specification, [the] immune effector cells are pharmaceutical composition[s] for treating cancer in animals, particularly humans." (Office action at page 5). The Examiner also alleges that "[t]he specification though is silent with respect to the effect of DC-TC hybrids on humans." (Id.). Applicants traverse.

Claims 35 and 45 have been cancelled herein. Thus, this rejection is moot with respect to these claims. Applicants assert that the as-filed specification provides numerous examples of the therapeutic effect achieved by education of immune effector cells (including cytotoxic T lymphocytes ("CTLs")) using dendritic cell-tumor cell hybrids in human cancer patients, including breast cancer patients and ovarian cancer patients. For example, Figure 6B show the cytolysis of autologous breast cancer tumor (BT) target cells by T cells educated by dendritic cell-breast cancer tumor cell fusion cells (DC/BT) (shaded circle) as compared with T cells educated by autologous BT cells (open circle). The replicated data were obtained with cells from three different breast cancer patients.

Further, Figure 7A shows the cytolysis of autologous BT cells or autologous monocytes (MC) by T cells stimulated with autologous DC/BT fused cells. The replicated data were obtained with cells from two different breast cancer patients. Similarly, Figure 7B demonstrates

the effect of an antibody specific for human MHC class I molecules on cytolysis by T cells educated by autologous DC/BT fused cells, or autologous BT cells, autologous MC, MCF-7 breast cancer cells, ovarian cancer cells (OVCA), and K562 cells. The presence of the anti-MHC class I antibody is indicated in the hatched bars and solid bars indicate the absence of the antibody. This result shows that T cells educated by autologous DC/BT fused cells are superior to T cells educated by unfused cells in their ability to kill tumor cells, and that disruption of the MHC class I molecule diminishes this ability.

Additionally, Figure l0B shows the cytolysis of autologous OVCA target cells by T cells educated by ovarian cancer-dendritic cell (OVCA/DC) fused cells (shaded circle) as compared to autologous DC (open circle), autologous OVCA cells (open box), or autologous OVCA cells mixed with DC (open triangle). The data were obtained with cells from three different ovarian carcinoma patients. These results demonstrate that dendritic cell-tumor cell hybrids have the superior capacity to educate T cells that are cytotoxic to tumor cells, as compared to unfused cells. Similarly, in Figure l0C, cytolysis of autologous human ovarian cancer (OVCA) target cells by T cells educated by OVCA/DC fused cells (OVCA/FC) is demonstrated to be superior to that observed using T cells stimulated with autologous DC, autologous OVCA cells, autologous monocytes (MC), autologous monocytes fused to autologous DC (DC/MC), or autologous OVCA cells fused to autologous monocytes (OVCA/MC).

Moreover, Figures 11A-B and 12A-B present similar data, which demonstrates the cytotoxic effect of educating T cells with tumor cell-dendritic cell hybrids as compared to educating T cells with unfused cells.

Therefore, Applicants contend that the as-filed specification does provide numerous examples of the therapeutic effect that is achieved in human cancer patients by education of immune effector cells using dendritic cell-tumor cell hybrids. Thus, this rejection should be withdrawn.

The Examiner has also stated that "the specification only teaches producing CD8+ CTL cells and fails to show that the hybrid cells indeed expanding the population of CD4+ cells (working examples)." (Office action, page 5). Applicants traverse.

The instant specification explicitly discloses that both CD4+ and CD8+ T cells are expanded by contact with the fused cells of the invention. For example, the expansion of CD4+ and CD8+ T cells is demonstrated by the *in vivo* depletion of immune cell subsets (*i.e.*, CD4+

and CD8+ T cells) using specific antibodies, which is described in the as-filed specification at, *e.g.*, page 49, lines 6-12, which recites:

> "[t]o further define the effector cells responsible for antitumor activity, mice were injected intraperitoneally with antibodies against $CD4^+$ or $CD8^+$ cells before and after immunization with FC/MUC1. Depletion of the respective population by 80-90% was confirmed by flow cytometric analysis of splenocytes. The finding that injection of anti-CD4 and anti-CD8 antibodies increases tumor incidence indicated that both $CD4^+$ and $CD8^+$ T cells contributed to antitumor activity. Moreover, depletion of $CD4^+$ and $CD8^+$ T cells was associated with reduced lysis of MC38/MUC1 cells *in vitro*."

See also Figure 2, which also demonstrates the expansion and role of both CD4+ and CD8+ T cells educated by the fused cells of the invention. Therefore, contrary to the Examiner's assertion, the specification clearly teaches that the hybrid cells expand the population of CD4+ cells. Thus, this rejection should be withdrawn.

Applicants also assert that the Examiner has mischaracterized the post-filing Parkhurst publication, which was submitted with the Response and Amendment filed October 1, 2003. The Examiner states that "*Parkhurst et al* teach that even though DC-tumor hybrids have been shown to have a protective effect in animal tumor models, in the recent two clinical trials, fusions of DCs and autologous tumors are primarily *ineffective* for treating tumor[s] in humans." (Office action, paragraph bridging pages 5-6).

However, Applicants note that Krause *et al.*, 2002 J. Immunother. 25:421-8 ("Krause") (copy of abstract supplied herewith) and Kikuchi *et al.*, 2001 Cancer Immunol. Immunother. 50:337-44 ("Kikuchi") (copy of abstract supplied herewith), the two publications cited in the Parkhurst reference that discuss clinical trials of dendritic cell-tumor cell hybrids generated by researchers other than the Applicants, do not support the Examiner's contention that the pending claims are not enabled. Moreover, these hybrids cannot be compared to the hybrid cells of the present invention.

For example, the methods used to generate the hybrid vaccine by Krause (autologous monocyte-derived dendritic cells were fused with gamma-irradiated primary autologous tumor cells by incubation with PEG; *see* Krause, Abstract), differ from the methods used in the present invention, which do not require irradiated tumor cells, but instead provide, in certain embodiments, the irradiation of the hybrid cells. Likewise, Krause does not teach that the fusions of dendritic cells and autologous tumors are primarily ineffective for treating tumors in

humans. Instead, Krause states that "a hybrid vaccine of DC and tumor cells can be safely applied and can induce tumor regressions," but that the clinical efficiency of the approach in the form used by the authors of the Krause study is insufficient (Krause, Abstract).

Similarly, the work described in Kikuchi can be distinguished from the hybrid cells of the present invention because the fusion efficiency of the present invention (30-50% in breast cancer cell-dendritic cell fusions and 32.6% in ovarian cancer cell-dendritic cell fusions; *see, e.g.,* Specification page 58, lines 23-25; and page 62, lines 7-10) is superior to the Kikuchi fusion efficiency (21.9%; *see*, Kikuchi, Abstract). Thus, neither Krause nor Kikuchi teach that the fused cells of the present invention will not educate immune effector cells.

The Examiner further states that, giving the broadest reasonable interpretation to the claims, the immune effector cells encompass B cells, monocytes, macrophages, NK cells, and any T lymphocyte (*e.g.*, CD3+, CD4+, or CD8+). (*See*, Office action at page 6). Moreover, the Examiner also asserts that since B cells, NK cells and macrophages are terminally differentiated cells, they are "unlikely to become CD4+ or CD8+ T cells upon contacting" with the hybrid cells of the invention, and, as such, the pending claims are not enabled. (Id.).

In response, Applicants note that independent claims 32 and 42 have been amended herein to require the limitation that immune effector cells are T lymphocytes. The specification teaches that " 'T-lymphocytes' denotes lymphocytes that are phenotypically CD3+, typically detected using an anti-CD3 monoclonal antibody in combination with a suitable labeling technique." (Specification at page, 9, lines 25-27). The specification also teaches that dendritic cells provide all of the signals required for T cell activation and proliferation, including the interaction between the T-cell receptor/CD3 ("TCR/CD3") complex and an antigenic peptide presented by a major histocompatibility complex ("MHC") class I or II protein on the surface of an antigen-presenting cell, such as the fused cells of the invention. (*See*, *e.g.*, Specification at page, 10, lines 20-23).

Therefore, the as-filed specification discloses methods by which T lymphocytes are educated by dendritic cell-containing fusion cells. Thus, one of ordinary skill in the art would be able to practice the invention as presently claimed without undue experimentation.

For each of the above-stated reasons, Applicants assert that the pending claims are fully enabled. Thus, this rejection should be withdrawn.

Claim Rejections -- 35 U.S.C. § 102

1. Claims 32, 35-39, 42, 45-49, 52-55, 84, 89 and 90 have been rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,306,388 ("Nair I") or U.S. Patent No. 6,387,701 ("Nair II") in view of Janeway Jr., *Immunobiology*, 2001 ("Janeway"), Hural *et al.*, J. Immunol. 2002 ("Hural"), and Powell *et al.*, J Immunother Emphasis Tumor Immunol. 1995 ("Powell"). Claims 35 and 45 have been cancelled herein. Thus, this rejection is moot with respect to these claims. Applicants traverse this rejection to the extent it applies to the pending claims, as currently amended.

In the Response and Amendment filed October 1, 2003, Applicants demonstrated that the population of CD4+ immune effector cells and CD8+ immune effector cells of the present invention, which are educated by hybrid cells generated by fusion of mammalian dendritic cells and mammalian tumor or cancer cells, are distinct from the immune effector cells described by Nair I, which are educated by antigen presenting cells (APCs) into which exogenous RNA has been introduced. Specifically, the population of immune effector cells educated by contact with the antigen producing cells generated by the Nair I method will be CD8+, but will not be CD4+.

The Examiner did not find this argument persuasive and noted that "there is no evidence that the RNA loaded-APCs would only stimulate CD8+ T cells, and because the nature of an immune response is determined by the type of the antigens, the state of the antigen-presenting cells, and the cell population contacted with the activated APCs." (Office action, paragraph bridging pages 7-8). The Examiner cites Nair II in support of this position, stating "in the newly cited patent, *Nair et al* clearly teach that the APCs used for loading the tumor RNAs express MHC class II molecules, thus, the subsets of T cells generated contains both CD8+ and CD4+ cells." (Office action, page 9). Applicants traverse.

Applicants believe that the Examiner's reliance on Nair II is misplaced. Nair II states that preferred APCs such as dendritic cells and macrophages express MHC class II molecules. (Nair II, col. 4, lines 1-2). However, as outlined by Dr. Kufe in the attached Declaration ("Kufe Declaration II"), the ability of an antigen presenting cell to express MHC class II molecules does not, by itself, lead necessarily to stimulation of CD4+ cells, in the absence of exogenous ligands endocytosed by the APC. Kufe Declaration II, ¶9. Rather, the manner in which the antigen is introduced into the antigen presenting cell, along with the type of antigen itself, determines

which of the two possible pathways of antigen processing and presentation are taken by a given antigen. Id.

The Examiner also indicates that Janeway "teaches that tumor antigens could induce both CD8+ and CD4+ T cell responses," and that Hural teaches "that naturally processed CD4 T cell epitopes are present in the prostate-specific (tumor) antigen." (Office action at page 8). Thus, the Examiner concludes that "many tumor antigens could associate with MHC class II molecules and induce CD4+ T cell response." (Id.). Dr. Kufe indicates the Examiner's reliance on the statements of Janeway and Hural as evidence that the antigen presenting cells of Nair I would necessarily educate both CD8+ T cells and CD4+ T cells is incorrect. Kufe Declaration II, ¶10.

According to Dr. Kufe, antigen presentation occurs via the MHC class I-restricted pathway or the MHC class II-restricted pathway. Specifically, an antigen may enter a pathway of degradation leading to peptide presentation with MHC class I molecules in one of three ways: i) endogenously synthesized proteins are degraded and presented with MHC class I; ii) proteins injected into cytoplasm or fused with the plasma membrane enter the pathway; or iii) protein components of phagocytosed, nucleated cells also enter the MHC class I pathway. Kufe Declaration II, ¶7. In contrast, only exogenous antigens that are endocytosed by the antigen presenting cell are presented with MHC class II molecules. Kufe Declaration II, ¶7. (*See also,* Carbone and Bevan, "Major Histocompatibility Complex Control of T Cell Recognition," pp. 541-67, in Fundamental Immunology, 2nd ed., W.E. Paul, ed., 1989; courtesy copy enclosed). Antigens presented on an antigen-presenting cell via the MHC class I pathway will educate CD8+ immune effector cells, while antigens presented on an antigen-presenting cell via the MHC class II pathway will educate CD4+ immune effector cells. Kufe Declaration II, ¶7. Therefore, Dr. Kufe concludes that a given antigen, presented via the MHC class I-restricted pathway or the MHC class II-restricted pathway, will educate CD8+ T cells or CD4+ T cells, but not both. Id.

Thus, the Nair I methods, by which tumor polypeptides are introduced to the antigen presenting cells, cannot activate both the MHC class I and MHC class II pathways, as discussed above. Janeway and Hural do not rebut this argument. Rather, these references indicate that various tumor polypeptides, such as tyrosinase and PSA, contain peptide antigens that could associate with MHC class II molecules, if they were provided as exogenous antigens that are endocytosed by the antigen presenting cell. Nair I does not teach or suggest the introduction of

exogenous antigens that are endocytosed by the antigen presenting cell. Moreover, even assuming, arguendo that Nair I did, the educated T cells would only be CD4+. Kufe Declaration II, ¶10.

The Examiner also alleges that the starting material of Nair I "would already include both cell population[s] in the Nair reference, thus, the end product would meet [the] claim limitation." (Office action, page 9). Once again, the Examiner cites Janeway and asserts that this reference teaches that T cells obtained from peripheral blood mononuclear cells (PBMC) are either CD4+ cells (two-thirds of the cells) or CD8+ cells (one-third of the cells). However, the pending claims specify that the immune effector cells are "educated, antigen-specific immune effector cells." As Dr. Kufe notes, the CD4+ cells and CD8+ cells found in the Nair I starting material cited by the Examiner would not be specific for the tumor antigen, as required by the pending claims. Kufe Declaration II, ¶11.

Thus, for each of the above-stated reasons, Applicants contend that Nair I does not anticipate the claims as amended herein. Therefore, this rejection should be withdrawn.

2. Claims 32, 35, 37, 42, 45, 47, 52-55, 84, 89 and 90 have rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,156,307 ("Granucci") in view of Janeway Jr., *Immunobiology*, 2001 ("Janeway"), Hural *et al.*, J. Immunol. 2002 ("Hural"), and Powell *et al.*, J Immunother Emphasis Tumor Immunol. 1995 ("Powell"). Claims 35 and 45 have been cancelled herein. Thus, this rejection is moot with respect to these claims. Applicants traverse this rejection to the extent it applies to the pending claims as currently amended.

In the Response and Amendment filed October 1, 2003, Applicants distinguished the population of CD4+ immune effector cells and CD8+ immune effector cells of the present invention, which are educated by hybrid cells generated by fusion of mammalian dendritic cells and mammalian tumor or cancer cells, from the immune effector cells taught by Granucci. Moreover, according to Dr. Kufe, Granucci teaches that antigens can be associated with MHC class I or MHC class II molecules of an antigen presenting cell. Kufe Declaration II, ¶13. Thus, the resulting population of immune effector cells educated by contact with these APCs will be either CD8+ or CD4+, but not both. Id.

The Examiner has admitted that this either-or distinction exists, stating "*Granucci et al* is not limited to tumor antigen[s] but embraces any antigen which can be associated with either

class I molecule[s] such as viruses and bacteria, or as associated with class II molecule[s] such as other bacteria and parasites." (Office action, paragraph bridging pages 10 and 11, emphasis added). However, the Examiner cites Janeway and Hural as teaching "that tumor antigens could induce both CD8+ and CD4+ T cell responses." (Office action at page 11). As Dr. Kufe states in his Declaration, Janeway and Hural merely teach that various tumor polypeptides, such as tyrosinase and PSA, contain peptide antigens that could associate with MHC class II molecules if they were provided as exogenous antigens, which would initiate the MHC class II pathway, or if they were provided via one of the methods discussed above to enter the MHC class I pathway. *See* Kufe Declaration II, ¶13. Thus, Applicants contend that neither Janeway nor Hural teach or suggest that a tumor antigen (or any other antigen) could induce both CD8+ and CD4+ responses. Therefore, the pending claims are not anticipated by Granucci in view of Janeway or Hural.

The Examiner also argues that the starting material of Granucci, like that of Nair I, "would already include both cell population[s] in [the] *Granucci* reference, thus, the end product would meet [the] claim limitation." (Office action, paragraph bridging pages 11-12). However, as noted above, the pending claims recite that the immune effector cells are "educated, antigen-specific immune effector cells." Thus, Dr. Kufe notes that the CD4+ cells and CD8+ cells found in the Granucci starting material cited by the Examiner would not be specific for the tumor antigen, as required by the pending claims. *See* Kufe Declaration II, ¶14. Therefore the starting material taught by Granucci does not contain all the limitations of the pending claims, as amended herein.

Therefore, for each of these reasons, Applicants contend that the educated antigen-specific immune effector cells of the pending claims are functionally and patentability distinct different from the immune effector cells educated by the loaded APCs of Granucci. Thus, Granucci does not anticipate the claims as amended herein, and this rejection should be withdrawn.

Claim Rejections -- 35 U.S.C. § 103

Claims 32, 36, 38, 39, 42, 46, 48 and 49 have also been rejected under 35 U.S.C. § 103(a) as being obvious over Granucci or Nair II in view of Altenschmidt *et al.* 1997, J. Immunol. 159:5509-15 ("Altenschmidt").

As discussed above, neither Granucci nor Nair II teach or suggest all of the limitations of the claimed invention. Altenschmidt has been cited by the Examiner for the teaching of genetically modifying immune effector T cells. (Office action, page 14). However, Altenschmidt does not cure the deficiencies of Granucci or Nair II, as there is no teaching or suggestion in Altenschmidt of an educated antigen-specific population of immune effector cells that comprises both $CD4^+$ immune effector cells and $CD8^+$ immune effector cells. According to Dr. Kufe, one of ordinary skill in the art seeking to overcome the limitations of Granucci or Nair II discussed above would not be motivated to use the genetic modification technique of Altenschmidt, since, as it was only applied to T cells, this technique would not result in the association of MHC class I and MHC class II molecules in the antigen presenting cell such as a dendritic cell. Kufe Declaration II, ¶16.

Therefore, because Granucci or Nair II in view of Altenschmidt fail to teach or suggest all of the limitations of the claimed invention, these claims are not obvious in view of these references. Thus, this rejection should be withdrawn.

CONCLUSION

Based on the instant amendments and remarks, Applicants submit that this application is in condition for allowance and such action is respectfully requested. Should any questions or issues arise concerning the application, the Examiner is encouraged to contact Applicants' undersigned attorney at the telephone number indicated below.

Respectfully submitted,

May 14, 2004

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS :	Gong and Kufe *(as amended herein)*		
SERIAL NUMBER :	09/782,492	EXAMINER :	Qian J. Li
FILING DATE :	February 12, 2001	ART UNIT :	1632
FOR :	CELL FUSIONS AND METHODS OF MAKING AND USING THE SAME		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

I, DONALD KUFE, hereby declare and state as follows:

1. I received my Doctor of Medicine degree in 1970 at the University of Rochester School of Medicine. I am a named inventor on this application. I have been working in the fields of immunology, cell and molecular biology and with methods of treating cancer since 1977.

2. I understand that the pending claims are directed to substantially pure cytotoxic populations of educated, antigen-specific immune effector cells expanded in culture by contacting these effector cells with hybrid cells that are generated by fusion between at least one mammalian dendritic cell and at least one mammalian tumor or cancer cell.

3. I am aware of the Examiner's December 18, 2003 Office Action. In particular, I understand that the Examiner has rejected the pending claims under 35 U.S.C. § 102(e) contending that the pending claims are anticipated in view of United States Patent No. 6,306,388 to Nair *et al.* ("Nair I") or United States Patent No. 6,487,701 ("Nair II"), and in view of United States Patent No. 6,156,307 to Granucci *et al.* ("Granucci"), taken in view of Janeway Jr., *Immunobiology*, 2001 ("Janeway"), Hural *et al.*, J. Immunol. 2002 ("Hural"), and Powell *et al.*, J Immunother Emphasis Tumor Immunol. 1995 ("Powell"). I understand the Examiner has also rejected the pending claims under 35 U.S.C. § 103(a) contending that the pending claims are obvious in view of Granucci or NairII in view of Altenschmidt *et al.* 1997, J. Immunol. 159:5509-15 ("Altenschmidt").

4. It is my understanding that the Examiner has taken the position that the RNA-loaded antigen presenting cells of Nair I are not limited to stimulating CD8+ T cells. The Examiner also takes the position that the antigen presenting cells of Granucci, and the combination of Granucci and Altenschmidt, can simultaneously induce both CD8+ and CD4+ T cell responses.

5. I make this declaration to rebut the Examiner's rejections, with which I do not agree. I understand that the claims of the instant application have been amended to specify that, in addition to requiring substantially pure cytotoxic populations of educated, antigen-specific immune effector cells expanded in culture by contacting these effector cells with hybrid cells that are generated by fusion between at least one mammalian dendritic cell and at least one mammalian tumor or cancer cell, the pending claims also require that this population contains both $CD4^+$ immune effector cells and $CD8^+$ cytotoxic immune effector cells, and that that the immune effector cells contacted with the hybrid cells are T lymphocytes.

6. The hybrid cells of the instant application process tumor-specific antigens via both the MHC class I and class II pathways. As such, the resulting population of immune effector cells contacted with the hybrid cells of the invention is cytotoxic to target tumor cells and contains $CD4^+$ immune effector cells and $CD8^+$ cytotoxic immune effector cells. Such cytotoxic populations of immune effector cells are neither taught nor suggested by Nair I, by Granucci, or by the combination of Granucci and Altenschmidt.

7. Antigen presentation can occur via the MHC class I-restricted pathway or the MHC class II-restricted pathway. An antigen may enter a pathway of degradation leading to peptide presentation with MHC class I molecules in one of three ways: i) endogenously synthesized proteins are degraded and presented with MHC class I; ii) proteins injected into cytoplasm or fused with the plasma membrane enter the pathway; or iii) protein components of phagocytosed, nucleated cells also enter the MHC class I pathway. In contrast, only exogenous antigens that are endocytosed by the antigen presenting cell are presented with class II molecules. (*See,* Carbone and Bevan, "Major Histocompatibility Complex Control of T Cell Recognition," pp. 541-67, in Fundamental Immunology, 2nd ed., W.E. Paul, ed., 1989; a copy

of which is provided herewith). Antigens presented on an antigen-presenting cell via the MHC class I pathway will educate CD8+ immune effector cells, while antigens presented on an antigen-presenting cell via the MHC class II pathway will educate CD4+ immune effector cells. Therefore, a given antigen, presented via the MHC class I-restricted pathway or the MHC class II-restricted pathway, will educate CD8+ T cells or CD4+ T cells, but not both.

8. The cytotoxic population containing both CD4+ immune effector cells and CD8+ immune effector cells of the present invention, is, in my opinion, distinguishable from and superior to the immune effector cells of Nair I. The Nair I immune effector cells are educated by antigen presenting cells (APCs) into which exogenous RNA has been introduced, and, thus, they will be CD8+, but not CD4+. I understand that the Examiner has disagreed with this argument, stating that "there is no evidence that the RNA loaded-APCs would only stimulate CD8+ T cells, and because the nature of an immune response is determined by the type of the antigens, the state of the antigen-presenting cells, and the cell population contacted with the activated APCs." (Office Action, paragraph bridging pages 7-8).

9. The Examiner cites Nair II in support of this position, stating that "in the newly cited patent, *Nair et al* clearly teach that the APCs used for loading the tumor RNAs express MHC class II molecules, thus, the subsets of T cells generated contains both CD8+ and CD4+ cells." (Office action, page 9). I believe that the Examiner's reliance on Nair II is misplaced and leads her to a faulty conclusion regarding the ability of the Nair I RNA-loaded APCs to generate both CD8+ T cells and CD4+ T cells. Nair II does state that preferred APCs such as dendritic cells and macrophages, express MHC class II molecules. (Nair II, col. 4, lines 1-2). However, it is well recognized in the art that the ability of an antigen presenting cell to express MHC class II molecules does not, by itself, necessarily lead to the stimulation or education of CD4+ cells in the absence of exogenous ligands endocytosed by the APC. Rather, the type of antigen, as well as the manner in which the antigen is introduced into the antigen presenting cell, determines which of the two possible pathways of antigen processing and presentation are taken by a given antigen. Thus, because exogenous RNA is introduced into APCs in the Nair methods, the resulting immune effector cells will be CD8+ and not CD4+.

10. The Examiner cites several additional references, none of which alter my opinion regarding the distinctions between the immune effector cells of the present invention and those described in Nair I. The Examiner states that Janeway "teaches that tumor antigens could induce both CD8+ and CD4+ T cell responses," and that Hural teaches "that naturally processed CD4 T cell epitopes are present in the prostate-specific (tumor) antigen." (Office action at page 8). Thus, the Examiner concludes that "many tumor antigens could associate with MHC class II molecules and induce CD4+ T cell response." (Id.). In my opinion, the Examiner's reliance on these statements from the Janeway and Hural references in order to support the contention that the Nair I antigen presenting cells would educate both CD8+ T cells and CD4+ T cells is misplaced. Moreover, it is also my opinion that the Nair I methods where tumor polypeptides are introduced to the antigen presenting cells by a single method (*e.g.*, loading of exogenous RNA encoding a tumor antigen), cannot simultaneously activate both the MHC class I and MHC class II pathways. The Examiner's reliance on Janeway and Hural does not alter my opinion, as these references merely indicate that various tumor polypeptides, such as tyrosinase and PSA, contain peptide antigens that could associate with MHC class II molecules, if they were provided as exogenous antigens that are endocytosed by the antigen presenting cell. I do not believe that Nair I discloses or suggests the introduction of exogenous antigens that are endocytosed by the antigen presenting cell. In any event, even if Nair I did teach or suggest such a method, the resulting T cell population would be CD4+ only.

11. The Examiner also indicates that the starting material of Nair I "would already include both cell population[s] in the Nair reference, thus, the end product would meet [the] claim limitation." (Office action, page 9). To this end, the Examiner again cites Janeway and asserts that this reference teaches that T cells obtained from peripheral blood mononuclear cells (PBMC) are either CD4+ cells (two-thirds of the cells) or CD8+ cells (one-third of the cells). While I agree that the population of T cells obtained from PBMC may contain T cells that are CD4+ or CD8+, the pending claims recite that the immune effector cells are "**educated, antigen-specific** immune effector cells." Clearly, the CD4+ cells and CD8+ cells found in the Nair I starting material would not be specific for the tumor antigen, as is required by the pending claims.

12. Thus, for all of these reasons, I believe that the population of cytotoxic immune effector cells of the instant application, which are generated by contact with hybrid cells formed by fusion of dendritic cells with cancer or tumor cells and contain both $CD4^+$ immune effector cells and $CD8^+$ cytotoxic immune effector cells, are functionally and patentably distinct from the immune effector cells disclosed by Nair I.

13. Additionally, in my opinion, the cytotoxic population of CD4+ immune effector cells and CD8+ immune effector cells of the present invention, is also distinguishable from and superior to the immune effector cells of Granucci. Granucci teaches that antigens can be associated with MHC class I or MHC class II molecules of antigen presenting cells. Thus, the population of immune effector cells educated by contact with these APCs will be either CD8+ or CD4+. (*See, e.g.*, Granucci col. 5, lines 19-34). The Examiner appears to agree that this distinction exists and states that "*Granucci et al* is not limited to tumor antigen[s] but embraces any antigen which can be associated with either class I molecule[s] such as viruses and bacteria, or as associated with class II molecule[s] such as other bacteria and parasites." (Office action, paragraph bridging pages 10 and 11, emphasis added). However, the Examiner also cites Janeway and Hural for teaching "that tumor antigens could induce both CD8+ and CD4+ T cell responses." (Office action at page 11). As noted, it is my belief that these references merely indicate that various tumor polypeptides, such as tyrosinase and PSA, contain peptide antigens that could associate with MHC class II molecules if they were provided as exogenous antigens, which would initiate the MHC class II pathway, or via one of the methods discussed above to enter the MHC class I pathway. These references do not support the Examiner's contention that a tumor antigen (or any other antigen) could induce both CD8+ and CD4+ immune effector responses.

14. The Examiner also alleges that the starting material of Granucci, like that of Nair I, "would already include both cell population[s] in [the] *Granucci* reference, thus, the end product would meet [the] claim limitation." (Office action, paragraph bridging pages 11-12). However, it is my understanding that the pending claims recite that the immune effector cells are "educated, antigen-specific immune effector cells." Clearly, the CD4+ cells and CD8+ cells

found in the Granucci starting material cited by the Examiner would not be specific for the tumor antigen, as required by the pending claims.

15. For each of these reasons, I believe that the population of cytotoxic immune effector cells of the instant application, which are generated by contact with hybrid cells formed by fusion of dendritic cells with cancer or tumor cells and which contain both $CD4^+$ immune effector cells and $CD8^+$ cytotoxic immune effector cells, are functionally and patentably distinct from the immune effector cells disclosed by Granucci.

16. It is also my opinion that the population of immune effector cells of the instant application are immunologically, biochemically, and patentably distinct from the combination of Granucci or Nair II and Altenschmidt. As indicated above, the antigen presenting cells disclosed by Granucci or Nair II can be loaded with antigens, which are associated with either Class I or Class II MHC molecules, but not with both. Altenschmidt discloses the genetic modification of T cells with a heterologous gene encoding a fragment of TCR and an anti-ErB2 receptor antibody. (*See, e.g.*, Altenschmidt, Abstract). It is my opinion that one of ordinary skill in the art seeking to overcome the deficiencies of Granucci or Nair II discussed above would not be motivated to use the genetic modification technique described in Altenschmidt, as doing so would not result in the association of tumor antigens with both MHC class I and MHC class II molecules in an antigen presenting cell such as a dendritic cell, since the target cell in Altenschmidt is the T cell, not the antigen presenting cell.

17. Thus, for all the foregoing reasons, it is my opinion that the pending claims are also not obvious over Granucci or Nair II in view of Altenschmidt. Therefore, the Examiner should withdraw this rejection and allow the pending claims.

18. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Donald W. Kufe

Donald Kufe

Signed at Boston, MASSACHUSETTS
this 12th day of May 2004

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